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Research Article

Effects of Letrozole and Metformin Versus Metformin Alone on Ovarian Activities and Gonadotropin-Releasing Hormone Receptor Antibodies (GnRh) in Iraqi Patients With Polycystic Ovarian Syndrome (PCOS)

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KEY WORDS:
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Abstract:
In recent times, an increasing emphasis has been placed on a novel category of autoantibodies. This particular group of autoantibodies specifically targets the receptors that are coupled to G protein (GPCRs), primarily focusing on the 2nd extracellular loop (ECL2). These autoantibodies have the ability to either trigger or block particular GPCR signaling pathways, thus playing a crucial role in the pathogenesis of numerous ailments such as PCOS. The goal of the research is exploring the effects of Letrozole and metformin versus the sole use of metformin on ovary activities and GnRh antibodies in Iraqi subjects complaining of PCOS. The current investigation enrolled seventy five women with an age range of 20-<39 years. Patients were categorized into three groups: metformin group (positive control group) and they received metformin as 500 mg per-oral (bid), letrozole group who were treated using 2.5 mg per-oral (bid) and combination groups who received both agents with similar doses as above. Each group included 25 female. Data about body mass index (BMI) and the age were included in the study. Serum measurement of GnRhR antibodies was done before treatment and 90 days after treatment using Enzyme-Linked Immunosorbent Assay. In addition, assessment of ovarian characteristics was done using ultrasound. Changes reveled the folowing: at baseline before treatment, there were no signficant diffrences among study groups (p = 0.983); it was observed that the giving of either item alone led to a considerable decrease in. level of GnRH antibody, however, bringing the two items in combination led to more notable decrease in its level (p<0.001). The metformin alone was able significantly to make larger the volume of dominant follicls (p = 0.040), Letrozole alone was also able significantly to to make larger the volume of dominant follicle at (p = 0.006), while used both drugs caused more significant increase (p<0.001). Changes. It was observed that resistive index was reduced following use of either drugs alone at (p<0.01), however, combined use of both drugs show more signficant reduction (p<0.001). Combined treatment with letrozole and metformin is safe and efficient in PCOS women leading to improvement of overall ovarian activity by reducing levels of GnRh receptors antibodies with possible synergistic effect.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a condition affecting the system of endocrine glands. that impacts around 4-20% of women in their reproductive years[1,3]. Diagnosis of Polycystic Ovary Syndrome (PCOS) according to the Rotterdam criteria necessitates the manifestation

of at least two out of the three specified biochemical and clinical parameters: excess androgen levels, an ovulation/oligo-ovulation and ultrasound-confirmed polycystic ovarian morphology[4]. PCOS is linked to various comorbidities such as hepatic steatosis, mood fluctuations, dyslipidemia, resistance to insulin, diabetes mellitus of type 2, eating disorders, metabolic syndrome, infertility, and obesity. Additionally, women with PCOS face an elevated likelihood of developing hypertension, gestational diabetes, premature delivery and miscarriages[5-11].

In normal ovulation females, gonadotropin-releasing hormone (GnRH) is secreted in a periodic fashion by hypothalamus, leading to pulsatile release of follicular stimulating hormone (FSH) and luteinizing-hormone (LH), which subsequently interact with the ovary to induce the process of ovulation[12]. Conversely, the disease (PCOS) is distinguished by a fluctuating increase in LH and testosterone with an unclear pathogenesis[13,14]. Efforts in the past to pinpoint a self-immune etiology in PCOS have concentrated on both the pituitary-hypothalamic axis and peripheral target tissues, yet these endeavors have yielded limited insights[15,16].

In recent times, an increasing emphasis has been placed on a novel category of autoantibodies. This particular group of autoantibodies specifically targets the receptors that are coupled to G protein (GPCRs), primarily focusing on the 2nd extracellular loop (ECL2). These autoantibodies have the ability to either trigger or block particular GPCR signaling pathways, thus playing a crucial role in the pathogenesis of numerous ailments such as PCOS[17-20].

Own-antibodies targeting GPCRs exhibit notable distinctions from endogenous ligands of GPCR in the effects on receptor activity. While natural ligand prompts desensitization of the receptor through homologous mechanisms, as observed in treatments involving GnRH or synthetic analogs like leuprolide, GPCR-specific autoantibodies do not induce such desensitization[21]. These autoantibodies are capable of directly stimulating their target GPCR., moreover, they have the ability to modulate the interaction between the GPCR and natural ligands in a positive or negative manner by binding allosterically to receptor structures not associated with the ligand-binding pocket[22,23]. A recent study highlighted a notable increment in GnRhR antibodies in individuals with PCOS contrasted to a reference cohort, underscoring the important implications for etiology, diagnosis and treatment brought about by their presence[17]. The current investigation was aiming at exploring the effects of Letrozole and metformin versus metformin alone on ovarian activities and GnRh antibodies in Iraqi women complaining of PCOS.

MATERIALS AND METHODS

The current investigation enrolled seventy five women with an age range of 20<40 years. Those patients were labeled as having the disease (PCOS) based on criteria of Rotterdam (Rotterdam, 2004) by 2 specialists in obstetrics and gynecology. Pregnant women, women with co-morbidities such as diabetes mellitus, essential hypertension, liver disease and kidney disease, women with hyperprolactinemia and women with thyroid disease were not allowed to take part in the study. Patients were categorized into three groups: metformin group (positive control group) and they received metformin as 500 mg per-oral (bid)., letrozole group who were treated using 2.5 mg per-oral (bid) and combination groups who received both agents with similar doses as above. Each group included 25 women. The patients were recruited from the Maternity and Pediatrics Teaching Hospital in Adiwaniyah Province, Iraq. The research is dated back to October the 21st 2023 and extended to March 31st 2024.

Data about body mass index (BMI) and the age were included in the study. Serum measurement of GnRhR antibodies was done before treatment and 90 days after treatment using Enzyme-Linked Immunosorbent Assay (ELISA) (BT LAB, China). In addition, assessment of ovarian characteristics was done using ultrasound. The research was granted approval by the ethical review board of the College of Medicine at the University of Al-Qadisiyah. All participants were informed to give a written consent after complete explanation of the procedures and goals of the current study.

Statistical work was managed using (SPSS version 26.0, IBM, Chicago, USA). Numeric data were shown in the form of standard deviation, range and mean. One way ANOVA test was used to contrast average values among study cohorts, which was followed y least significant difference post hoc test. The significance statistical level was set at p-value of less than or equaling 0.05.

RESULTS AND DISCUSSIONS

Comparison of demographic characteristics among study groups is shown in (table 1). There was a lack of notable disparity in the average age (p = 0.981) among study groups and means of age were 29.24±6.10 years, 29.56±5.50 years and 29.44±5.83 years, respectively and the range of age was between 18 and 39 years. In

Table 1: Comparison of demographic characteristics among study groups

Characteristic	Group M n = 25	Group L n = 25	Group ML n = 25	p
Age (years)				
Mean ±SD	29.24 ± 6.10	29.56±5.50	29.44 ± 5.83	0.981 O
Range	18-39	18-38	19 - 38	NS
BMI (kg/m^2)				
Mean ±SD	28.80 ± 2.72	27.53 ± 1.95	27.07 ± 1.99	0.534 O
Range	21.07-30.42	24.34-31.18	23.82-30.81	NS

O: one way ANOVA
n: number of cases
SD: standard deviation
NS: not significant
BMI: body mass index

Table 2: Changes in serum GnRh Ab levels after treatment

GnRh pg/ml	Group M n = 25	Group L n = 25	Group ML n = 25	P
Before treatment				
Mean ±SD	983.3 ± 213.6	1108.8 ± 391.6	1056.9± 350.5	0.983 O
Range	786.7-1201.2	865.7-1369.4	623.8-1259.8	NS
After treatment				
Mean ±SD	568.51 ± 191.5	493.8 ± 164.8	390.7 ± 125.8	0.049 O *
Range	237.3-827.5	351.6-687.9	262.8-922.6	
P	0.007 pa **	= 0.001 pa ***	< 0.001pa ***	

***:** significant at p = 0.05
O: one way ANOVA
n: number of cases;
SD: standard deviation
Pa: paired t-test
NS: not significant

Table 3: Changes in mean number of dominant follicles after treatment

Number of dominant follicles	Group M n = 25	Group L n = 25	Group ML n = 25	P
Before treatment				
Mean ±SD	11.19±1.15	10.76±1.24	11.43± 1.08	0.138 O
Range	9.36-12.83	8.55-12.11	8.78-12.63	NS
After treatment				
Mean ±SD	15.19 ± 0.62	16.88 ± 0.40	19.75 ± 0.91	0.031 O *
Range	14.0-15.9	16.1-17.5	18.3-21.6	
P	0.040 pa *	0.006 pa **	< 0.001pa ***	

NS: not significant
***:** significant at p = 0.05
****:** significant at p = 0.01
*****significant at p =0.001
SD: standard deviation
n: number of cases
Pa: Paired t-test
O: one way ANOVA

Table 4: Changes in mean resistive index after treatment

Resistive index	Group M n = 25	Group L n = 25	Group ML n = 25	P
Before treatment				
Mean ±SD	0.92 ± 0.09	0.90 ± 0.10	0.94 ± 0.08	0.153 O
Range	0.80-1.13	0.79-0.99	0.83-1.1	NS
After treatment				
Mean ±SD	0.81 ± 0.04	0.78 ± 0.03	0.69 ± 0.02	0.029 O *
Range	0.76-0.88	0.75-0.85	0.65-0.76	
P	0.004 pa **	0.006 pa **	< 0.001pa ***	

NS: not significant
***:** significant at p = 0.05
****:** significant at p = 0.01
*****significant at p =0.001
SD: standard deviation
n: number of cases
Pa: Paired t-test
O: one way ANOVA

addition, in this study, There was a lack of notable disparity in mean BMI (p = 0.534) among study groups and means of BMI were 28.80±2.72 kg/m^2, 27.53±1.95 kg/m^2 and 27.07±1.99 kg/m^2, respectively and the range of BMI was between 21.07 and 31.18 kg/m^2.

Changes in serum GnRh antibody are shown in (Table 2). At baseline before treatment, there were no signficant diffrences among study groups (p = 0.983). It was observed that giving any of the drugs alone led to considerable decrease in level of GnRH antibody;

however, use of the two items in combination led to more significant reduction in its level ($p<0.001$). Changes in mean number of dominant follicles are shown in table (3). The metformin alone was able significantly to increase the count of dominant follicles ($p = 0.040$), Letrozole alone was also able significantly to to increase the number of dominant follicles at ($p = 0.006$), while used both drugs caused more significant increase ($p<0.001$). Changes in mean resistive index are shown in table 4. It was observed that resistive index was reduced following use of either drugs alone at ($p<0.01$), however, combined use of both drugs show more signficant reduction ($p<0.001$).

The etiology of PCOS is incompletely understood; however, recently it has been suggested that autoimmunity may play a crucial role[17]. Autoantibodies to GnRh receptors have been indentified in women with PCOS and the serum level of these self-antibodies was greater in a significant manner in comparison with women with no PCOS based on recent reports[17]. Additionally, a published report has indicated the presence of autoantibodies targeting the second extracellular loop of the receptor of the gonadotropin-releasing hormone (GnRH-R) in most samples of serum from individuals with polycystic ovary syndrome (PCOS) that were tested[24]. Furthermore, a recent experimental investigation using a rat model of PCOS demonstrated that heightened levels of GnRH-R autoantibodies exacerbated luteinizing hormone (LH) levels, inflammation and high androgen. These alterations are likely associated with the observed insulin resistance in peripheral tissues due to the suppression of signaling pathway of the insulin-stimulated IRS/PI3K/Akt/Glut[25]. In this study, we made a suggestion that treatment of PCOS using commonly used pharmacological agents such as metformin and letrozole may act via their effect on these auto-antibodies. Infertility in women with PCOS is a common health problem and a substantial research work is directed to solve this issue using a variety of pharmacological approaches. Crucial to fertility potential is the quality of ovum and arterial resistance in female genital tract[26]. Thus, we correlated changes in GnRH-receptor antibodies to changes in number of dominant follicles and changes in arterial resistive index as assessed by ultrasound measurement before and after treatment.

We observed significant improvement in number of dominant follicles and resistive index concomitant with significant reduction in GnRH-receptor antibodies and these changes were significantly better using combination of metformin and letrozole in comparison with using either drug alone. We therefore, can suggest that both pharmacological agents affected antibodies levels and resulted in improvement in pituitary-gonadal axis hormonal action and therefore, the ovarian function got improved. Another suggestion can be made, that both agents may improve overall inflammatory response in women with PCOS and this improvement has been reflected as improvement in overall ovarian function. To the best of our knowledge, this is the first study that tested the effect of treatment using metformin and letrozole on anti-bodies directed against GnRH receptors in women with PCOS and this is the point of originality of this article. Further clinical and experimental research is needed in order to validate the results of the current study.

CONCLUSION

Combined treatment with letrozole and metformin is safe and efficient in women with PCOS resulting in improvement of overall ovarian activity by reducing levels of GnRH receptors autoantibodies with possible synergistic effect.

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